Phytochemistry, 1980, Vol. 19, pp. 535–537. © Pergamon Press Ltd. Printed in England.

ASYMMETRIC INCORPORATION OF 4-(2′-CARBOXYPHENYL)-4-OXOBUTYRATE INTO PHYLLOQUINONE BY *ZEA MAYS*

KENNETH G. HUTSON and DAVID R. THRELFALL

Department of Plant Biology, University of Hull, Hull, HU6 7RX, U.K.

(*Received* 26 *June* 1979)

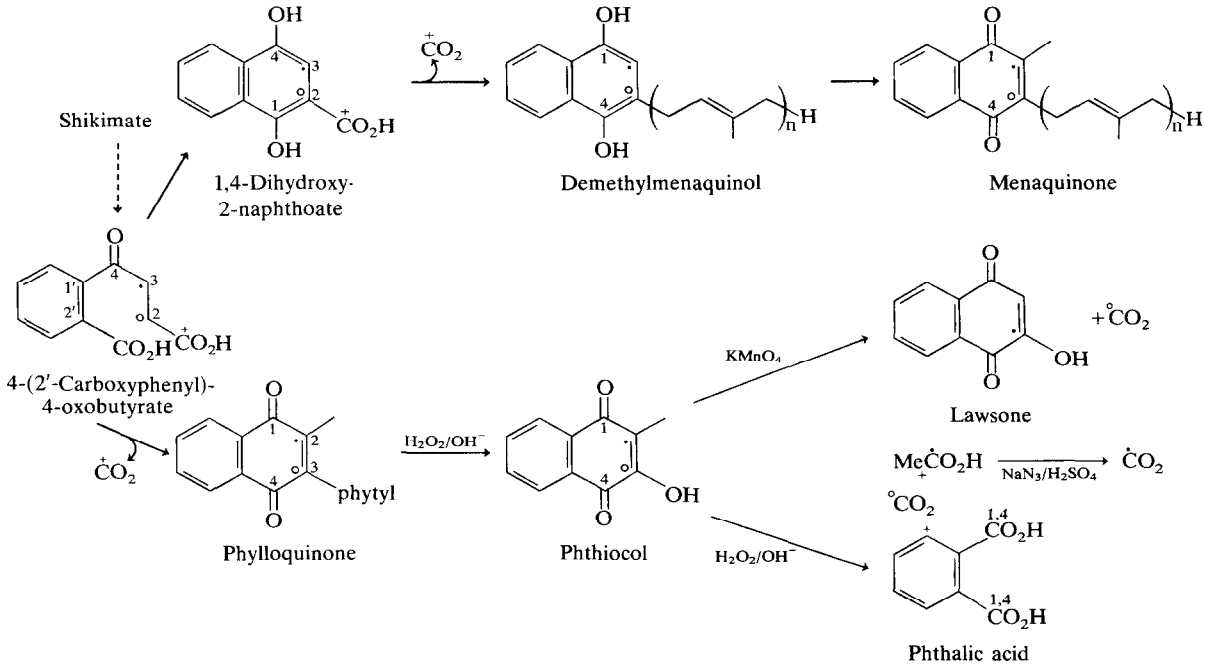
Key Word Index—*Zea mays*; Gramineae; maize; biosynthesis; phylloquinone; 4-(2′-carboxyphenyl)-4-oxobutyrate.

Abstract—Radioactivity from 4-(2′-carboxyphenyl)-4-oxobutyrate-[2-^{14}C] and 4-(2′-carboxyphenyl)-4-oxobutyrate-[3-^{14}C] was incorporated into C-3 and C-2 respectively of phylloquinone in maize shoots. These results show that this substrate is incorporated in the same asymmetric manner into phylloquinone as it is into the bacterial menaquinones.

INTRODUCTION

The aromatic ring, the C-4 carbonyl group, C-3, C-2 and the C-1 carbonyl group of the naphthaquinone nuclei of the bacterial menaquinones are derived from the phenyl ring, the C-2′ carboxyl group, C-2, C-3 and the C-4 carbonyl group respectively of 4-(2′-carboxyphenyl)-4-oxobutyrate (2-succinylbenzoate) (Scheme 1) [1, 2]. The asymmetric incorporation is brought about by virtue of the fact that the C-1 carboxyl group of the substrate is retained up until the biosynthetic step on the pathway at which 1,4-dihydroxy-2-naphthoate is decarboxylated and polyprenylated to form 2-polyprenyl-1,4-naphthaquinol (2-demethylmenaquinol) (Scheme 1) [3, 4].

In a previous paper, it was shown that 4-(2′-carboxyphenyl)-4-oxobutyrate is incorporated into the naphthaquinone nucleus of phylloquinone in a similar manner to that just described for menaquinones [5]. It was not possible, however, to determine if the incorporation took place in an asymmetric fashion [5] because the degradation procedures used to determine



Scheme 1. Outlines of the biosynthetic pathways for the incorporation of 4-(2′-carboxyphenyl)-4-oxobutyrate into phylloquinone and menaquinone and of the chemical degradation of the naphthaquinone ring of phylloquinone.

the pattern of labelling of phylloquinone-[^{14}C] samples labelled from different species of 4-(2′-carboxyphenyl)-4-oxobutyrate-[^{14}C] did not distinguish between C-1 and C-4, and between C-2 and C-3 of the phylloquinone nucleus.

We now present evidence to show that 4-(2′-carboxyphenyl)-4-oxobutyrate is incorporated in an asymmetric manner into the naphthaquinone nucleus of phylloquinone, and that as in the case of the bacterial menaquinones the 2′-carboxyl group of the substrate gives rise to the C-4 carbonyl group of the product.

RESULTS AND DISCUSSION

The ^{14}C-labelled phylloquinones were isolated from the controls of an experiment designed to investigate the effect of homocysteine on the synthesis of phylloquinone and dehydrophylloquinone in etiolated maize shoots exposed to light [Hutson, K. G. and Threlfall, D. R., unpublished work]. Chemical degradation of the ^{14}C-labelled phylloquinones by the procedures outlined in the Scheme 1 showed that 82–91% of the radioactivity in the molecule was associated with C-2 when 4-(2′-carboxyphenyl)-4-oxobutyrate-[3-^{14}C] was the substrate and that 91% was associated with C-3 when 4-(2′-carboxyphenyl)-4-oxobutyrate-[2-^{14}C] was the substrate (Table 1).

When considered in conjunction with the results of the previous study which showed that the C-1 carboxyl group of 4-(2′-carboxyphenyl)-4-oxobutyrate is not incorporated into phylloquinone in maize shoots and that the phenyl ring, the C-4 and C-2′ carboxyl groups, and C-2 and C-3 of this substrate give rise to the aromatic ring, the C-1 and C-4 carbonyl groups, and C-2 and C-3 respectively of phylloquinone [5], the results of the present study show that 4-(2′-carboxyphenyl)-4-oxobutyrate is incorporated into the naphthaquinone nucleus of phylloquinone in the same asymmetric manner as it is incorporated into the naphthaquinone nuclei of bacterial menaquinones (see Introduction and Scheme 1).

EXPERIMENTAL

Radiochemicals. 4-(2′-Carboxyphenyl)-4-oxobutyrate-[2-^{14}C] (8.2 mCi/mmol) and 4-(2′-carboxyphenyl)-4-oxobutyrate-[3-^{14}C] (14.5 mCi/mmol) were synthesized from 2-carboxybenzaldehyde and the appropriate ^{14}C-labelled species of sodium pyruvate (Radiochemical Centre, Amersham, Bucks, U.K.) by the method of Dansette [2]

Exposure of maize shoots to radiosubstrates. Shoots (100) of etiolated 7-day-old maize seedlings (*Zea mays* cv Kelvedon) were excised at the node and the cut ends dipped into 30 ml 0.05 M Pi buffer, pH 6.5, containing 2 μCi of radiosubstrate They were exposed to continuous illumination (800 lm/ft^2) for 20 hr at 28°.

Extraction and purification of phylloquinone. This was carried out as described in ref. [6].

Chemical degradation of phylloquinone. Procedures followed (Scheme 1) were based on those described in refs. [2] and [5].

Radioassay. Samples were assayed for radioactivity by liquid scintillation counting. All counts were corrected for background, quenching and instrument efficiency.

Acknowledgements—This work was supported by the SRC. We thank Mrs. Susan Swetez for expert technical assistance.

REFERENCES

1. Baldwin, R. M., Snyder, C. D. and Rapoport, H. (1973) *Biochemistry* **13,** 1523.

Table 1. Chemical degradation of ^{14}C-labelled phylloquinones

Degradation products	4-(2′-Carboxyphenyl)-4-oxobutyrate 2-^{14}C		3-^{14}C	
	(10^{-3} dpm)	(dpm/μmol)	(10^{-3} dpm)	(dpm/μmol)
^{14}C-Phylloquinone	1082	ND*	371	ND*
↓				
^{14}C-Phylloquinone + carrier	541	1754	247	801
↓				
^{14}C-Phthiocol	165	1732 (99)†	87	796 (99)
↓ ~¼ ; ↘ ⅖ Lawsone	0.51	68 (4)	4.68	730 (91)
^{14}C-Phthiocol + carrier	38	162	18	76
↓				
Phthalic acid	0	0	0	0
+				
CO_2	35 (91)	—	1 (5)	—
+				
NaAc	1.59	8 (5)	16	70 (91)
↘ CO_2	—	—	14.4 (82)‡	—

* Yield of purified ^{14}C-phylloquinone in the range 0.2–0.4 μmol.

† Figures in parentheses give % total activity in ^{14}C-phylloquinone.

‡ Part of sample lost.

2. Dansette, P. (1972) Docteur és-Sciences Physiques These, Université de Paris-Sud, Centre d'Orsay.
3. Young, I. G. (1975) *Biochemistry* **14,** 399.
4. Bryant, R. W. and Bentley, R. (1976) *Biochemistry* **15,** 4792.
5. Thomas, G. and Threlfall, D. R. (1974) *Phytochemistry* **13,** 807.
6. Threlfall, D. R. and Whistance, G. R. (1977) *Phytochemistry* **16,** 1903.